FORM-REGULATION IN CERIANTHUS, IV.

The Rôle of Water-pressure in Regeneration.

C. M. CHILD.

Introductory.

The regeneration of the marginal tentacles in cylindrical pieces was described in the first paper of this series (Child, '03*a*).

The tentacles do not arise from the new tissue closing the end, nor from the cut surface, but from the old body-wall itself, which first becomes thinner and loses its muscular layer in the region where the tentacles are to appear and then gives rise to small buds, corresponding in number and position to the intermesenterial chambers.

The fact that the new marginal tentacles arise some distance away from the cut surface by a local transformation of the already differentiated body-wall is of considerable importance. There must be some adequate ground for the localization of this peculiar process of transformation in a region of the body-wall which apparently differed in no way from adjacent parts before the cut was made.

Very early in my study of regeneration in *Cerianthus* it became evident that the rapidity of regeneration was more or less closely connected with the degree of distension of the piece. My observations along this line were made chiefly upon *C. solitarius*. In cases where closure of the ends and consequently distension by water was possible regeneration was much more rapid than in pieces where communication between the enteron and the exterior — other then the mouth and the aboral pore — existed. Observation of this apparent relation between water-pressure and regeneration led to experiment and it was soon possible to demonstrate that a very close relation between water-pressure in the enteron and regeneration existed. I am aware, of course, that Loeb ('91, '02) regards certain phases of this relation in *Cerianthus* as osmotic in nature. The results of his experiments and his interpretation will be discussed at another time: it need only be said here that Loeb failed to understand the real conditions, otherwise he would scarcely have attempted to apply the osmotic hypothesis to this case.

The evidence bearing upon the relation between regeneration and internal water-pressure is varied in character. In the remaining portion of the present paper and in following papers the various lines of evidence will be discussed. First, however, it is necessary to call attention to certain features of the water-pressure and circulation in the enteron of normal animals. Except where statement to the contrary is made all observations and experiments were made upon *C. solitarius*.

Water-pressure and Circulation in the Enteron.

Under ordinary conditions both the body-wall of *Cerianthus* and the tentacles are subjected to a considerable degree of tension in consequence of internal water-pressure, the enteric cavity being distended with water. When contraction occurs this water issues through the aboral pore, at least in large part, though some passes out through the stomodæum. After loss of water from the enteron the body and tentacles are shorter and smaller, and, if the loss was great, are more or less completely collapsed. This condition continues until the enteron again begins to fill with water. As it fills, the body gradually resumes the original form exactly as does a rubber balloon when inflated with air. This distension of body and tentacles by water under pressure is an indispensable condition of the characteristic form of body and tentacles. The animal is incapable of extending to its full length or attaining its full diameter in any other way than through the medium of the internal water-pressure. Extension of the tentacles and the turgid condition often designated as erection is just as completely the result of the enteric water-pressure. Turgor of individual cells has absolutely nothing to do with the condition, as is evident to all who have examined with any care *Cerianthus* or other actinians. Loeb's belief ('91) that osmosis plays a part here is wholly without foundation, except so far as water may diffuse through the body-wall into the enteron.

We may regard *Cerianthus* as a sac filled with water under pressure. Under certain conditions the walls of the sac may decrease in size either locally or at all points, the result being in the first case increase of pressure and either stretching of other parts of the sac or loss of water through the pore or mouth, and in the second case always loss of water, commonly through the pore, and reduction in size of the whole body. While this sac has the power of contracting and forcing water out through certain openings, it has not the power of expanding actively and so drawing water in.

Since water under pressure is normally present in the enteron, and since after collapse due to contraction the pressure is soon reëstablished, means must exist by which water can be forced into the enteron against pressure. In my first paper (Child '03*a*) mention was made of the fact that regenerating pieces closed at both ends by their membranes of new tissue become distended with water in the course of a few days. It was suggested that in such cases water diffuses through the body-wall in consequence of the accumulation within the enteron of certain soluble products of metabolism. It is also possible that water may be secreted into the enteron together with these substances. To what degree the distension in normal animals is caused in this manner cannot be definitely ascertained, but it is certain that the rapid accumulation of water in the enteron of the normal animal after this has lost all or nearly all of its contents in consequence of violent contraction cannot be due to diffusion or secretion. In such cases the animal frequently regains its usual degree of distension within fifteen or twenty minutes, whereas distension by diffusion in regenerating pieces requires several days.

The few observations which have been made upon the siphonoglyphes in actinians indicate that the function of these structures is the production of currents through the stomodæum. Hickson ('83) found that in certain Alcyonaria, which possesses only one siphonoglyphe, the current was inward or downward along the siphonoglyphe and outward on the other portions of the stomodæal surface. It is probable further, according to Hickson, that the inward or downward current is continued along the free edges of the mesenteries for a greater or less distance below the siphonoglyphe, while on other mesenterial margins the cilia beat in the reverse direction, like those of the general stomodæal surface outside the siphonoglyphe.

According to this view there is an inward and an outward current in the stomodæum and for a greater or less distance aboral to it in the central region of the enteron between the margins of the mesenteries. According to my own observations conditions are very similar in *Cerianthus*. Only one siphonoglyphe is present (Fig. 1) and along this the current is directed inward, though whether the cilia may ever reverse this movement has not determined. The fact that small particles placed on the disc, if not ingested by muscular action, are gradually carried in a peripheral direction indicates that here the cilia beat so as to cause an outward current. The direction of the current on the lower parts of the œsophagus outside of the siphonoglyphe has not been determined, but is probably the same as upon the disc and upper portions. In Fig. 2 the opposing stomodæal currents are indicated by arrows, the inward current along the siphonoglyphe by the longer arrow on the left and the outer currents by two shorter arrows on the right.

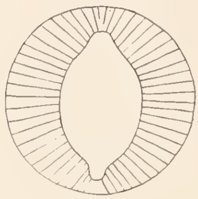

Fig. 1.

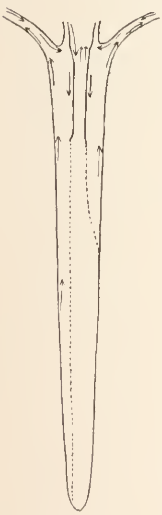

Fig. 2.

The natural conclusion is that the accumulation of water in the enteron is in some way the resultant of the stomodæal currents, but the manner in which this result is attained requires consideration. When the enteron is distended with water under pressure the walls of the œsophagus must be brought into contact since pressure is exerted upon them from all sides by the water in the enteron. The radiating grooves corresponding to the lines of attachment of the mesenteries, which are visible upon the disc of *Cerianthus*, extend the whole length of the stomodæum and probably it is along these that the chief outward currents pass. As the walls of the œsophagus are pressed more more closely together these grooves are more and more nearly obliterated. Probably when internal pressure is high the outward currents are very slight or absent. The siphonoglyphe, however, is a relatively powerful organ, which produces an inward current. This groove is probably always open whether the body is distended or not. According to the laws of fluid pressure the force of the inward current need be only slight since the pressure it exerts is transferred to every unit of surface and in all directions in the enteron, *i. e.*, the principle by which enteric pressure is maintained is that of the hydraulic press ; a slight pressure exerted over a very small surface is transferred to a large body of confined fluid and so multiplied many times.

If these suggestions are correct the passage of water in and out of the body and the process of distension and collapse are accomplished somewhat as follows : When the body is contracted it is probable that the œsophagus is more or less widely open ; observation of living animals or sections of contracted specimens (Fig. 1) support this view. This condition of the œsophagus is probably brought about by contraction of the mesenteries which possess a slightly developed transverse musculature. In the contracted state then there is free passage of water in and out. When the muscles relax the œsophageal walls are again brought into more or less close contact, this result being, perhaps, brought about in part by the elasticity of the œsophageal walls or by muscular contraction, and in part by the circulatory currents in the intermesenterial chambers on all sides of the œsophagus (see Figs. 1 and 2). The approximation of the œsophageal walls decreases the outward currents, while the inward current continues, since the siphonoglyphe remains open. Thus, more water enters than passes out, and the result is of course gradual distension of the body. As the internal pressure increases the walls of the œsophagus are more and more closely appressed and thus the outward currents are decreased still further while the inward current continues undiminished. The increase in pressure and distension will continue until the motive power exerted by the cilia of the siphonoglyphe is just sufficient to balance the internal pressure and prevent an outward movement of water, and to replace any loss through outward currents, if such still continue, as perhaps they do to a slight extent. So long as the various conditions remain unchanged distension continues.

Let us now suppose that a sudden stimulus leads to contraction of the body-muscles. In the first stages of this contraction the internal pressure must be greatly increased and probably the œsophageal walls are more closely appressed than before, since the muscles which separate them are weak. At this stage then little or no water escapes through the œsophagus. When the pressure reaches a certain point the aboral pore is forced open and rapid ejection of water occurs with considerable force. Thus the internal pressure is relieved, and now, as further contraction occurs, separation of the œsophageal walls takes place, and the remaining water passes out of the œsophagus, sometimes carrying with it mesenterial filaments in case of violent contraction. The point to which I desire to call especial attention is that when contraction begins the internal pressure closes the œsophagus all the more tightly, and it is not until this pressure is relieved by escape of water through the aboral pore that opening of the œsophagus can take place. Though few observations have been made on other species of actinians, I am inclined to believe that distension and collapse may be accomplished in much the same manner in other members of the group, the cinclides or other openings taking the place of the aboral pore. It is probable, however, that in many forms the muscles which separate the œsophageal walls are more powerful than in *Cerianthus* and so are able to bring about separation in spite of the internal pressure. It is perhaps needless to state that the ejection of mesenterial filaments through the cinclides or mouth is purely passive, the filaments being merely carried out with the water.

One other point may be mentioned in this connection: if the preceding observations and suggestions are correct it follows that the form of the œsophagus usually observed in transverse sections, viz., an oval with grooves at one or both ends (Fig. 1) is not what might be called the natural form, but a form resulting from contraction. Practically all fixed specimens of actinians are more or less contracted. It is extremely probable that when the animal is distended in the normal manner the œsophageal walls are always closely appressed except in the region of the siphonoglyphe. Dilation occurs of course in the taking of food, but it probably does not extend over the whole length of the œsophagus at one time and so does not cause any great loss of water. That the œsophagus is widely open in contracted actinozoa is very evident from the frequent forcing out through it of mesenterial filaments; that it cannot be widely open during distension of the body by water is equally evident. Doubtless many differences in detail occur in different members of the group, but it is probable that the suggestions given here for *Cerianthus* will apply more or less closely to a large number of forms. The presence of two siphonoglyphes must condition certain modifications in these processes. The statement is made in several text-books that the siphonoglyphes serve to keep the water in circulation when the animal is contracted. According to the view given above, these organs serve rather to permit circulation when the body is distended.

In addition to the inward and outward currents in the stomodæal region the water in the intermesenterial chambers and in the tentacles connected with them is in constant circulation, impelled by the movement of cilia. I have not been able to determine the details of this circulation in *Cerianthus*, but have observed it in several transparent members of the group. In general it may be said that the current passes orally along the body-wall, peripherally along the aboral face of the tentacle, back again along its oral face, centrally beneath the disc, probably into and out of the labial tentacles, to the stomodæum, and aborally along the stomodæum. Whether cilia on the lateral surfaces of the mesenteries aid in forcing the water aborally I do not know. In the forms observed, at any rate, the aboral current continued from the aboral end of the stomodæum to the aboral end of the body. In all of the intermesenterial chambers are these circulatory currents. In *Cerianthus* itself they are difficult to demonstrate with certainty, but there is little doubt that the current in the oral direction along the body-wall exists, and this being present, we may confidently assert that the return current is also present. These currents in the intermesenterial chambers and tentacles may be designated for convenience as the circulatory currents in distinction from the inward and outward currents of the stomodæal region. They are indicated diagrammatically by arrows in Fig. 2.

How far the circulation of water in the central regions of the body is complicated by the complex arrangement of the mesenterial margins and mesenterial filaments cannot be determined. Moreover, these are merely matters of detail and do not affect the general outlines of the process. Since the position of the organs is subject to considerable variation the direction of local currents produced by their cilia must vary likewise.

This discussion of the internal circulation and pressure has been somewhat detailed, since I believe that the general internal pressure and perhaps also local pressure resulting from the impact of currents against the body-wall are important factors in form-regulation and without doubt also in development.

Internal Pressure, Growth and Form-regulation — A Preliminary Survey.

It is necessary before proceeding to the account of my experiments to indicate briefly how growth and form-regulation may be affected by the internal water-pressure. It would be difficult otherwise in many cases to show the bearing of the experiments without extended explanation.

It is conceivable that the presence of water in the enteron may affect the body-wall in two ways: first, by general pressure, the same in all parts of the body, which subjects the body-wall to tension; second, by local pressure resulting from the impact of definitely directed currents upon some portion of the body-wall which interrupts their course, thus subjecting that part of the body-wall to a localized tension in addition to the general tension.

The experiments to be described demonstrate clearly that regulation in *Cerianthus* is dependent in certain important respects upon the tension resulting from internal water-pressure. The tissues react to this tension by growth. In the absence of the tension the typical form of the animal does not appear. Up to this point the evidence afforded by the experiments can scarcely be doubted or refuted.

An essential feature in form-regulation in *Cerianthus*, as well as in other forms, is localized growth which is especially noticeable in the formation of new tentacles.

The problem of the cause of localized growth is one of the greatest importance in morphogenesis. According to one view the basis of morphological form is inherent in the organism, either in the structure of the protoplasm or as a governing principle distinct from physical and chemical factors to which the latter are subordinated. On the other hand, it is possible, at least theoretically, to regard organic form as the resultant of the complex of physical and chemical conditions internal and external which affect the protoplasm. According to this view, organic form is not strictly speaking inherent in the living substance; but results indirectly from its activities and its environment.

The experiments to be described demonstrate, I believe, that in the absence of internal water-pressure the living substance of *Cerianthus* is absolutely incapable of producing the typical form of this animal. It is not merely that the parts are formed and remain collapsed in the absence of water-pressure; either they are not formed or their form is atypical. The appearance of the tentacles is delayed or is inhibited; the growth of the disc and the œsophagus does not occur when the internal pressure is reduced below a certain point. Thus it is possible in this case to demonstrate a general relation between internal water-pressure and growth.

The question as to whether any relation between localized growth and localized internal pressure exists must not be confused with the preceding question as to a general relation between the two phenomena. It is possible for instance to suppose that the region where new tentacles shall appear is determined in some unknown manner, but that the internal pressure is a factor in causing their growth after their position has been determined. On the other hand, it is possible to conceive that the position of these structures is determined by local pressure due to currents in the enteron (the "circulatory currents"). It cannot be claimed for my experiments thus far that they decide which of these two possibilities is correct. Certain of the data, however, appear to me to indicate that the local pressure due to currents may play a certain rôle in determining the position of the marginal tentacles. Concerning the labial tentacles there is as yet no definite evidence and this fact must constitute a weak point in the evidence, at least until further experiments can be performed.

Time need be taken here only for a brief consideration of the possibility of a direct relation between local internal pressure and the determination of localized structures at certain points. In each intermesenterial chamber the circulatory current passing orally along the inner surface of the body-wall (Fig. 2) must strike against any part of the wall which is folded or rolled over in such a manner as to form an obstacle in its course. When a transverse cut in the body-wall of *Cerianthus* is made the cut edges roll inward in such a manner that the current passing orally in the chambers must strike the inner surface of the inrolled portion. In this condition that portion of each intermesenterial chamber just beneath the inrolled cut margin forms a blind sac into which water is continually being forced by the current. The cilia which in the normal animal provide for a return current have been in large part removed from this region. Local pressure upon the body-wall in this region must result. Now the marginal tentacles always make their appearance in exactly this region, a region which previously formed a part of the lateral body-wall, and which, since tentacle-regeneration is possible at any level except the extreme aboral region, cannot have possessed any special qualifications for tentacle formation. The question is, what causes tentacles to appear in this particular region of a piece, no matter what part of the body the original piece represents. Doubtless very few will regard the assumption of an "entelechy" (Driesch) or of "dominants" (Reinke) as an answer to this and similar questions, although it may possess the merit of many other unwarranted assumptions, viz., that of being an easy way out of difficulty. If we call in "heredity" to answer the question we still have no real answer, though it is evident that tentacles of characteristic form and structure would not appear if *Cerianthus* were not concerned. Are we not justified in seeking for definite, intelligible causes or conditions of one kind or another for phenomena of this nature? Much work of recent years has given an affirmative answer to this question. In many cases undoubtedly these causes or conditions are internal, *i. e.*, in the protoplasm, but it is by no means impossible or improbable that they are external in many other cases.

Certain of my experimental results seem to me not only to admit the possibility of local pressure as a determinative factor in morphogenesis but to afford little basis for other interpretations. I desire to give the data in descriptive form, but shall endeavor to point out their bearing in connection with this hypothesis. It should be borne in mind, however, that while the existence of a general relation between internal water-pressure and growth is demonstrated, my data upon the other problem are insufficient in my own opinion to establish a conclusion without doubt. At present, though the evidence in regard to the marginal tentacles is very strong, the impossibility of giving any special evidence concerning the labial tentacles constitutes a serious defect. The regeneration of labial tentacles is certainly delayed or inhibited by reduced water-pressure, but I have not been able thus far to demonstrate that localized pressure upon the body-wall occurs in the region where they appear. This failure is due at least in part to the fact that my attention was concentrated chiefly on the marginal tentacles during my experiments. I hope that a future opportunity for renewed experimentation may render it possible to attain more definite conclusions on this point. Attention may be called to the possibility that the two sets of tentacles possessing different functions and appearing as they do at different times and under different conditions may perhaps be determined by very different factors. General similarity of form and structure is not necessarily indicative of similar conditions of origin. Even if it should be demonstrated that the marginal tentacles arise in response to the stimulus of localized pressure it would by no means necessarily follow that the labial tentacles are similarly produced.

The Closure and Distension of Pieces.

The closure by new tissue of the two ends of cylindrical pieces has been described in the first paper of this series (Child, '03*a*). The closure consists essentially in typical cases of the connection of all parts of the inrolled cut surface of the body-wall by a thin, delicate membrane of new tissue. The result of closure in this manner of both ends of the piece is the interruption of direct communication between the enteric cavity and the exterior. Nevertheless, water rapidly accummulates within the enteron, probably in consequence of diffusion or secretion, causing marked distension of the piece. However the fluid may enter, the distension occurs in every case, and in three or four days after section (in summer) the pieces are subjected to a considerable degree of internal pressure.

The pressure of the water being exerted in all directions tends to stretch the body-wall. The thin delicate new tissue at the ends is the weakest part of the body-wall and therefore most effected by the stretching. After closure of the ends by new tissue and before distension has occurred, the new tissue is either invisible or scarcely visible from the exterior. The inrolled edges are closely approximated and all that can be seen of the new tissue in any case is the part filling in the spaces between folds and wrinkles of the cut surface. Moreover, if the inrolled margins of the body-wall be spread apart even with the greatest care at this stage the new tissue does not stretch to any great extent but is ruptured even by slight tension. Under these conditions the cut

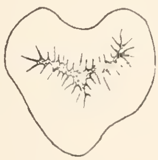

Fig. 3.

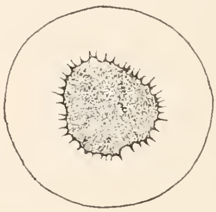

Fig. 4.

end presents somewhat the appearance of Fig. 3. The piece is collapsed, the margins are much folded and the only new tissue visible is the small area between the inrolled margins represented in the figure by the stippled portion.

These facts serve to introduce the chief point in the present consideration, viz., this: so long as there is no tension exerted upon this new tissue closing the ends it does not increase in surface-area, but mere differentiates into the typical structure of the body-wall. In other words the ends of the piece have no power in themselves to spread apart and thus initiate the formation of a new disc. What occurs in the typical course of regeneration is shown in Fig. 4. The inrolled margins are gradually spread apart while the thin membrane of new tissue uniting them — the stippled area in the figure — increases in area as they separate.

It is not difficult to show that this increase in area of the new tissue is due simply to the internal water-pressure. If the aboral end of the piece be prevented from closing or be punctured from time to time and thus the acclumulation of water in the enteron be prevented, the spreading of the oral end and the increase in area of the new tissue does not occur. The cut surfaces remain rolled inward and approximated as in Fig. 3, while the new tissue merely fills the spaces between them, but does not increase farther. A similar experiment affords the same result for the aboral end. In such cases the end retains the form acquired by inrolling of the margins (see Child, '03*a*, Figs. 3–5), differing from the stages figured only in that the crevices between the inrolled cut surfaces are closed by the new tissue.

Experiments of this kind were performed many times and always with the same result. Under conditions to be described later certain other regenerative processes may occur as usual in pieces which are kept open aborally, but in no case does the spreading apart of the cut surfaces at the oral end occur and in no case does the disc assume its typical form.

The increase in the area of the new tissue when under tension is not merely a stretching; as was noted above, attempts to stretch the tissue artifically always resulted in rupture. At the end of this extension of the new tissue its area is many times as great as at first and, what is more important, its thickness is as great as, or greater than before. Actual growth has without doubt occurred during the extension, indeed the conclusion is justified that the increase in area of the new tissue could not have occurred without growth; the attempts at artificial stretching leave little room for any other conclusion. If this conclusion is correct, then the growth of this new tissue is dependent on the tension to which it is subjected and not on any factor or condition existing in the tissue itself, except of course the power of growth. Unless the tissue is subjected to tension, growth ceases with the closure of the small spaces between the folds of the inrolled ends; when it is subjected to tension, within certain limits, it grows, *i. e.*, increases in quantity, number of cells, etc. Its increasing thickness and power of resistance to tension as differentiation occurs limit its growth, so that increase in area does not go beyond a certain point (Fig. 4).

In this case, as in the growth of new tissue between diverging cut surfaces described in the preceding paper (Child, '04), the localization and extent of growth is dependent, not on internal factors, but on simple mechanical conditions of tension.

The figures and the details of the description given refer to *C. solitarius*. The process of closure and distension in *C. membranaceus* differs in some details from this, though undoubtedly subject to similar conditions. It was shown in the preceding paper (Child, '04) that the new tissue in *C. membranaceus* is able to spread over larger areas and therefore to close larger openings than in *C. solitarius*, and moreover, that it is thicker and more resistant than in the latter species. Taking these facts into consideration we find that the process of closure and spreading of the ends is similar in both species. It is probable that the dependence of growth of the new tissue on tension would not have been so readily recognized in *C. membranaceus* if this form alone had been studied. The tissue after its formation is so resistant to tension that the distension of the piece is very commonly insufficient to bring about any considerable increase in the amount of new tissue after closure. Frequently, however, some growth after distension is recognizable, though it is usually relatively much less than in *C. solitarius*. This difference between these species indicates the importance of comparative study of related species in experimental work. Phenomena not readily interpreted in one species may be modified in another so that interpretation is without difficulty, and it is often possible, as in the present instance, to apply the interpretation obtained in the one case to the other, although its recognition in the first case would have been difficult or impossible.

Tentacle Regeneration in Relation to the Mesenteries.

Attention has already been called to the fact that tentacles do not regenerate in the absence of mesenteries (Child, '03*b*).

In regenerating ends representing levels near the oral end of the parent body the series of mesenteries is complete or nearly so ; farther aborally, however, mesenteries are present over only a larger or smaller portion of the circumference, according to the level. In the pieces from these regions a certain number of mesenteries regenerate and the series thus becomes complete. Still nearer the aboral end only a few mesenteries are present on the directive side, and finally only a single pair. In most of the pieces from this region regeneration of the mesenteries does not occur, probably because of the decreased reactive capacity of the tissues in this region, as has been suggested (Child, '03*b*). In certain pieces from this region the regeneration of tentacles corresponding to the intermesenterial chambers was observed to begin but never proceeded far and no mesenteries were regenerated (see Fig. 3, Child, '03*b*). The fact that tentacles never appear in such cases where mesenteries are absent indicates that their regeneration is dependent in some manner upon the presence of the mesenteries. In pieces where regeneration of the mesenteries occurs, regeneration of the tentacles never precedes or coincides in time with the appearance of mesenteries, but always follows. Various observations of others and my own upon other species of actinozoa indicate that this rule holds good not only for regeneration but for the normal development and for addition of new tentacles in adults. The new mesentery appears first, then the tentacle.

The interpretation of this "correlation" is not difficult if we accept the point of view suggested above. According to this it is evident that the mesenteries serve to localize the currents proceeding orally along the body-wall, *i. e.*, the current of one intermesenterial chamber is separated by the bounding mesenteries from that in the adjacent chamber. If the mesenteries were not present the current would strike the oral margin in a continuous circle and if it produced any effect at all would cause the outgrowth of the whole margin.[1]

The outgrowth of the whole margin, *i. e.*, the spreading of the disc, occurs to a certain extent in every case of regeneration and

[1] It is conceivable that in such a case the surface tension of the outgrowing margins might operate in such manner as to cause it to break up into a number of radiating masses of certain size. It is not impossible that the tentacles of certain cœlenterates are formed in some such manner whether the stimulus to growth be water-pressure or some other. This process might be compared to the breaking up of the margins of a drop of fluid into radiating cylindrical masses, which occurs when it is allowed to drop with force upon a smooth hard surface and "splashes." In this case we have a continuous cause with discontinuous effect. The close relation between the number of tentacles and the size, *i. e.*, the circumference, in such forms as *Hydra*, indicates the possibility of an explanation of this kind.

is probably due to the combined effect of the currents about the whole circumference, but the localized effect is much greater, giving rise to the long marginal tentacles. In its earlier stages the intermesenterial chamber formed between two regenerating mesenteries is simply a small blind pocket into which the water passing orally along the wall of the enteron is continually forced. In the normal animal there is a current passing aborally along the stomodæum (see Fig. 2), thus carrying the water out of the pocket, but in the earlier stages of regeneration the stomodæum and disc are absent; *i. e.*, no cilia, or few, are present to produce a current in the aboral direction. On the other hand the current passing orally is not necessarily diminished by section. The consequence is that water is continually forced into the oral end of the intermesenterial chamber and strikes the body-wall with a certain pressure, but no means for its removal exists except as the incoming water displaces it.

It is clear without further discussion how the localization of the regenerating tentacles and their dependence upon the presence of mesenteries can be accounted for as a reaction to the tension produced by internal water-pressure.

Local Inhibition of Tentacle Regeneration.

The shape of the collapsed pieces after section and before closure and distension is various. Frequently some part of the oral end — as well as other regions — becomes involved in some fold or wrinkle in consequence of collapse. The new tissue binds the cut surfaces together however they may happen to lie, but if distension occurs within a few days the folds are soon obliterated because the growth of the new tissue corresponds to the tension. If, however, distension be prevented by keeping open the aboral end while the oral end is permitted to close, the new tissue uniting the cut surfaces thickens and becomes more resistant to tension in the course of a few days.

After two weeks or more, even if the piece is allowed to close aborally and become distended, the fold may persist for a long time, since union of the oral end has occurred in the folded condition and the new tissue is now so resistant that only very gradual change occurs. It is easy to see that when such a piece becomes distended with water after closure it may be impossible or difficult for the water to gain entrance into the intermesenterial chambers of the folded part and so this may fail to be distended; if it is not distended it will be compressed to a greater or less extent by the adjacent distended regions. In this manner it is possible to produce experimentally cases in which only a part of the circumference becomes distended with water after closure. In all such cases it is found that the tentacles appear only on the distended portions. If the infolded or compressed portions become distended later, tentacles appear.

Provided the aboral end is kept open long enough, tentacles may appear in those regions where the mesenterial chambers are sufficiently open to permit the entrance and circulation of water. Of course the formation of tentacles is much slower in this case than when the piece is permitted to distend, and they never attain very great length. Since the regeneration of the tentacles is very slow and does not proceed far, this method is less satisfactory than the first.

By both of these methods it is possible to bring about local inhibition of tentacle-regeneration. In some cases the folded or compressed region includes only one or two mesenterial chambers, and here only one or two tentacles are inhibited or delayed. In pieces with slightly irregular oral margins where closure is irregular there is often great irregularity in the tentacles, some appearing early, others late, but in every case it can be determined that the tentacles appear first on those parts where distension and the circulation of water is least hindered, and *vice versa.*

There is very rarely any difficulty in distinguishing the distended portions of the body from those that are collapsed or compressed; the former appear somewhat translucent and the surface is smooth; the latter are opaque and usually more or less wrinkled. Moreover there is a distinct difference in color between distended and collapsed portions, the former being always lighter in color, since distension stretches the body-wall and the stripes (in *C. solitarius*) are farther apart or the pigment granules (in *C. membranaceus*) less densely distributed. Thus distended parts can usually be distinguished at a glance from those which are collapsed. It is possible to observe on the living specimens that the tentacle regeneration is more rapid in distended than in collapsed portions. Though it may not always be possible to determine exactly the causes that have operated in bringing about a particular case of local collapse or compression its presence can be recognized without difficulty, even before tentacle regeneration has begun.

A few examples selected from the numerous cases of this kind observed will serve to illustrate the subject. Fig. 5 represents a case in which one side of the oral region of a piece became folded inward during collapse and so failed to become distended when closure was allowed to occur. It will be observed that the new tissue closing the oral end does not form a rounded area but simply fills the crevices between the inrolled margins. The failure of the new tissue to distend is due to the fact that the aboral end was kept open until the new tissue had become thick

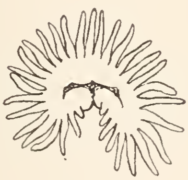

FIG. 5.

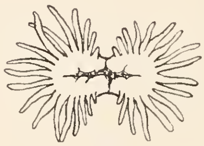

FIG. 6.

enough to resist the tension and to grow very slowly. It will also be observed that with approach to the infolded region the marginal tentacles decrease in size and finally cease. The region without tentacles represents a much greater portion of the circumference than is apparent in its contracted condition. The figure is simply a view of the oral end. A short distance from the end the fold disappears and the body is cylindrical in form. The fold was gradually obliterated in this piece and the missing tentacles finally appeared.

In Fig. 6 a similar case is shown, except that here two folds are present on opposite sides of the body. In the region of each of these folds the tentacles diminish in size and disappear as in the case of Fig. 5.

In these cases it is of course impossible to determine just why the water has failed to enter the infolded portions, but it is probable that the mesenteries of these parts are so closely pressed together that the mesenterial chambers are almost obliterated and their communication with the general enteric cavity almost or quite shut off. After the other parts have once become distended these collapsed parts are compressed. Only as water gradually finds its way into them, either by diffusion or through small openings, will they become distended so as to permit the typical circulation.

A case obtained in a similar manner from a piece of *C. membranaceus* is shown in Fig. 7 (natural size). It could be seen without difficulty that the three regions bearing the long tentacles were distended while the intermediate regions were not or were only slightly. In the course of a few weeks the distension of all parts gradually become uniform and the delayed tentacles finally attained the same size as the others, though only after the others had ceased to elongate. Of course at a somewhat earlier stage than that figured not even minute tentacle-buds were present on the infolded regions though the tentacles on the distended regions were already well developed. In this case as in the others it is probable that in the infolded regions the mesenteries are so closely pressed together that little water enters the chambers.

Fig. 7.

These experiments have a certain bearing upon the question of localized pressure as a factor in determining the position of the marginal tentacles. It is evident that in the distended portions the circulatory currents must possess greater force and volume than in the collapsed portions, where they may indeed be absent or insufficient to act as stimuli. If the appearance of the marginal tentacles is due to the local pressure resulting from these currents it is easy to see why the tentacles appear earlier on the distended portion. In general internal pressure we can find no factor which can determine the position of these organs, and if they are determined by "protoplasmic" factors it is difficult to understand why they should appear earlier on the distended portions than on the others.

Summary.

1. Except when the animal is contracted or collapsed the body-wall of *Cerianthus* is subjected to a certain degree of ten-

sion in consequence of the fact that the water in the enteron is under pressure.

2. The inward current along the siphonoglyphe is probably the means by which the internal pressure is established and maintained.

3. When the body is distended the œsophagus, with the exception of the siphonoglyphe and perhaps some grooves and crevices, must be closed.

4. When contraction occurs the water first issues from the aboral pore (in other actinians from the cinclides, etc.); then when the pressure is sufficiently reduced to permit it, the œsophageal walls are separated by muscular action and the remaining water issues from the mouth, often accompanied by mesenterial filaments. Thus the œsophagus is widely open only during extreme contraction.

5. The cilia on the entodermal surface of the body-wall produce a current flowing orally in each mesenterial chamber. The water passes from each chamber along the aboral face of the marginal tentacle, back on its oral face beneath the disc toward the stomodæum, probably into and out of the labial tentacles and aborally along the stomodæum. In all probability cilia along the sides or margins of the mesenteries force it further aborally.

6. The internal water-pressure plays a large part in form-regulation in *Cerianthus.* The general pressure affects the rapidity of growth wherever it may be taking place and it is possible that the local pressure exerted on the body-wall by the currents passing orally in each mesenterial chamber is the formative stimulus for the marginal tentacles.

7. Regeneration of tentacles is impossible unless mesenteries are present. The reason suggested for this is that in the absence of mesenteries there is no localization of the currents corresponding to the intermesenterial chambers, and, moreover, the water being unconfined between mesenteries, exerts less pressure on the inrolled oral end than if mesenteries were present.

8. Local retardation or inhibition of tentacle-regeneration can be brought about by preventing distension of a part or parts of the oral region.

Hull Zoölogical Laboratory,
University of Chicago, September, 1903

BIBLIOGRAPHY.

Child, C. M.

'03a Form-Regulation in Cerianthus, I. The typical Course of Regeneration. Biol. Bull., Vol. V., No. 5, 1903.

'03b Form-Regulation in Cerianthus, II. The Effect of Position, Size, and other Factors upon Regeneration. Biol. Bull., Vol. V., No. 6, Vol. VI., No. 1, 1903.

'04 Form-Regulation in Cerianthus, III. The Initiation of Regeneration. Biol. Bull., Vol. VI., No. 2, 1904.

Hickson, S. J.

'83 On the Ciliated Groove (Siphonoglyphe) in the Stomodæum of the Alcyonarians. Phil. Tr. Roy. Soc., Vol. 174, 1883.

Loeb, J.

'91 Untersuchungen zur Physiologischen Morphologie der Thiere. I. Ueber Heteromorphose. Wurzburg, 1891.

Loeb, J.

'02 Zusammenstellung der Ergebnisse einiger Arbeiten über die Dynamik des thierischen Wachsthums. Arch. f. Entwickelungsmech., Bd. XV., H. 4, 1903.